PROTEIN TECHNOLOGIES INTERNATIONAL
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July 9, 1999

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Dockets Management Branch (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Room 1061
Rockville, MD 20852

RE: Proposed Health Claim Linking Consumption of Soy Protein And Coronary Heart Disease; 63 Fed.Reg. 62977 (November 10, 1998)

Dear Sir or Madam:

Protein Technologies International, Inc. takes this opportunity to supplement its earlier comments and its original petition by providing a copy of a recently published study by Washburn, et.al. This study "Effect of Soy Protein Supplementation on Serum Lipoproteins, Blood Pressure, and Menopausal Symptoms in Perimenopausal Women", was recently published in The Journal of The American Menopause Society. This study reconfirms that daily consumption of 20g soy protein results in significant reductions of total cholesterol and low density lipoprotein cholesterol.

As demonstrated by the new studies submitted by the American Soybean Association and this study, the scientific data overwhelmingly supports FDA's tentative conclusion that a relationship exists between consumption of soy protein and reduced risk of CHD. We urge FDA to expedite publication of a final rule so that food manufacturers can convey this important dietary health information to consumers on food labels.

Sincerely,

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Cc: Susan Pilch

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Effect of Soy Protein Supplementation on Serum Lipoproteins, Blood Pressure, and Menopausal Symptoms in Perimenopausal Women

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ABSTRACT

Objective: To investigate the effect of soy protein supplementation with known levels of phytoestrogens on cardiovascular disease risk factors and menopausal symptoms in perimenopausal women.

Methods: A randomized, double-blind crossover trial was conducted in 51 women consuming isocaloric supplements containing 20 g of complex carbohydrates (comparison diet), 20 g of soy protein containing 34 mg of phytoestrogens given in a single dose, and 20 g of soy protein containing 34 mg of phytoestrogens split into two doses. Women were randomly assigned to one of the three diets for 6-week periods and subsequently were randomized to the remaining two interventions to determine whether differences existed between the treatment diets for cardiovascular disease risk factors, menopausal symptoms, adherence, and potential adverse effects.

Results: Significant declines in total cholesterol (6% lower) and low density lipoprotein cholesterol (7% lower) were observed in both soy diets compared with the carbohydrate placebo diet. A significant decline in diastolic blood pressure (5 mm Hg lower) was noted in the twice-daily soy diet, compared with the placebo diet. Although nonsignificant effects were noted for a number of measures of quality of life, a significant improvement was observed for the severity of vasomotor symptoms and for hypoestrogenic symptoms in the twice-daily group compared with the placebo group. No significant effects were noted for triglycerides, high density lipoprotein cholesterol or frequency of menopausal symptoms. Adherence was excellent in all groups.

Conclusions: Soy supplementation in the diet of nonhypercholesterolemic, nonhypertensive, perimenopausal women resulted in significant improvements in lipid and lipoprotein levels, blood pressure, and perceived severity of vasomotor symptoms. These data corroborate the potential importance of soy supplementation in reducing chronic disease risk in Western populations. (*Menopause* 1999;6:7–13. © 1999, The North American Menopause Society.)

Key Words: Hormone replacement therapy – Soybeans – Nutrition – Clinical trial – Menopause – Chronic disease prevention.

It is well recognized that enormous differences in chronic disease risk exist between populations residing in the Pacific Rim of Asia compared with those residing in Western Europe and North America.[1–3] A lower prevalence has been observed in Asian, compared with western countries for cardiovascular disease, reproductive cancer, and the presence of menopausal symptoms.[4–7] In addition, migrants from lower-to-higher chronic disease risk areas (i.e., migrants from Asian to western countries) have been observed to manifest the increased risk of their adopted country.[8–10] Although a large number of environmental factors may contribute to these differences in chronic disease burden, substantial differences in the consumption of soy-based products containing phytoestrogens have been postulated as one potential mechanism. Dietary soy supplementation significantly reduced lipids and lipoproteins

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in hypercholesterolemic subjects.[11–13] In addition, soy supplementation has been associated with an improvement in menopausal symptoms.[14–16] Many of the previous studies have been limited by inadequate assessment of phytoestrogen levels in the soy-based treatment, by not being randomized blind trials or by having relatively small sample sizes. This report seeks to determine the effect of a soy protein dietary supplementation with precisely known amounts of phytoestrogens on chronic disease risk and menopausal symptoms in a double-blind randomized crossover trial of nonhypercholesterolemic perimenopausal women with reported vasomotor symptoms.

METHODS

Sample selection

Participants in this trial were perimenopausal women aged 45–55 years. Eligibility requirements included presence of menopausal symptoms (at least one hot flush or night sweat daily), not currently using hormone replacement therapy (or using HRT in the past 6 months), and currently perimenopausal (missing at least three menstrual periods in the last 12 months and having last menstrual period not >12 months before participating in the study). Women were recruited through advertisements in a local newspaper. A total of 51 women agreed to participate in this study.

Study design

In this double-blind crossover clinical trial, women were randomly assigned to the order (i.e., first, second, or third 6-week period) of receiving the following 3 dietary supplements: (1) group 1: 20 g complex carbohydrate supplement (containing no phytoestrogens), (2) group 2: 20 g soy protein supplement (containing 34 mg of phytoestrogens consumed once daily), and (3) group 3: 20 g soy protein supplement (containing 34 mg of phytoestrogens split into two equal doses consumed twice daily).

The supplements were given to participants in a powder contained in individual daily packets. Women were encouraged to mix the powder with a beverage (milk or orange juice) and were also given a recipe book to allow them to consume the powder in other ways (i.e., mixed with yogurt, cereal, or with different types of beverages). The carbohydrate and soy protein supplements were provided for the study by Protein Technologies International, St. Louis, Missouri. The specific composition of the packets was assessed and is presented in Table 1. Both participant and clinical staff were unaware of the supplement type consumed by the participant. To assure study masking, the supplement was provided in identical appearing packets labeled with a study code (A, B, or C). Neither participants nor any investigators or clinical staff were informed of the coding scheme. No participant nor investigator/clinic staff member were unblinded during the course of the study.

Data collection

Before randomization, we obtained a baseline assessment of the frequency-severity of menopausal symptoms, health-related quality of life, physical measurements (blood pressure, height, and weight), and laboratory measures (lipids, lipoproteins, and clinical chemistry). These measurements were repeated at 6-week intervals to assess the impact of the three interventions. After 6 and 12 weeks, women were crossed over randomly to the other intervention diets. Thus, evaluation of these participants occurred at baseline, 6 weeks, 12 weeks, and 18 weeks to allow for assessment of impact of these interventions on chronic disease risk factors and menopausal symptoms.

Lipid and lipoprotein levels (total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, and triglyceride levels) were assessed using enzymatic techniques in the Wake Forest University Lipid Lab.[17] The Friedewald equation was used to indirectly calculate LDL cholesterol.[18] In addition to internal laboratory quality assurance measures, this laboratory also participates in the Centers for

TABLE 1. *Composition of the dietary supplements used in the clinical trial*

Ingredient	Soy protein supplementation (20 g protein) (%)	Carbohydrate placebo supplement (20 g carbohydrate) (%)
Moisture	2.5	2.7
Protein	68	<1.0
Carbohydrate	17	94.0
Sugars	12	48.0
Fat	3	<1.0
Saturated fat	0	0.0
Ash	9.5	1.1
Kilocalories	108.3	83.6
Vitamin A (IU)	531.0	555.7
Vitamin D (IU)	118.0	112.6
Vitamin C (mg)	2.66	2.62
Vitamin B1 (mg)	0.12	0.11
Vitamin B2 (mg)	0.53	0.46
Vitamin B6 (mg)	0.15	0.13
Vitamin B12 (mcg)	0.89	1.01
Niacin (mg)	0.00	0.24
Pantothenic acid (mg)	0.91	0.88
Folic acid (mcg)	59.0	13.8
Zinc (mg)	0.89	1.10
Calcium (mg)	708.0	2.2
Potassium (mg)	203.6	59.4
Sodium (mg)	203.6	7.9

Disease Control standardization program. Clinical chemistry measurements were obtained in the North Carolina Baptist Hospital Clinical Laboratory using an automated instrument (Bayer Chemistry, Tarrytown, NY). The study was conducted in the Wake Forest University School of Medicine General Clinical Research Center, which provided some of the nursing support staff and the nutrition staff, which provided both nutritional assessment and counseling for the trial. Three seated, resting blood pressure levels were obtained after a 5-min rest. Height (inches) and weight (kg) were measured to the nearest 0.1 unit with participants in light clothing, using a stadiometer and balance beam scale, respectively. Participants were asked to report the frequency of vasomotor symptoms (hot flushes and night sweats) and also to grade the severity of symptoms from mild to severe. In addition, a health-related quality of life questionnaire was administered at baseline and at the end of each 6-week intervention period. Using the health-related quality of life questionnaire data, an overall symptom score was calculated by using the Likert Scale information for the following content specific areas: estrogenic symptoms, general health, sleep disturbances, and gastrointestinal symptoms. Specifically, the estrogen symptom score was calculated to provide a continuous estimate of the intensity/frequency of symptoms known to be estrogen-dependent (e.g., vasomotor symptoms, vaginal dryness, sleep disturbances, breast tenderness, mood swings, and difficulties in concentration) with a higher score representing a greater estrogenic effect because of either increased frequency or severity of symptoms.

Statistical methods

Descriptive data are presented to describe the baseline characteristics of the participants. Data were analyzed to determine whether differences in chronic disease risk factors, menopausal symptoms, and adherence/side effects were present among the three treatment periods, and comparisons were made using data collected at the end of the respective treatment periods. The effects of diets were estimated and tested using a repeated measures general linear model that accounted for order effects.[19]

Study adherence

Adherence to the dietary supplements and to the overall study was excellent. Approximately 95% of the expected number of packets were consumed by participants, with no significant differences in adherence rates noted between the intervention groups. Nine women dropped out of the study shortly after randomization and did not complete a 6-week visit (five had personal reasons that prevented them from continuing to participate, one had an unrelated illness, one had concern about using artificial sweeteners (aspartame), one remembered that she was allergic to soy after 1 week in the study, and 1 had a recurrence of acne rosacea). Although the numbers were small, baseline characteristics were similar between the women who dropped out compared with the women who were longer term adherents.

RESULTS

Table 2 shows the baseline characteristics of the participants. These participants were perimenopausal, predominately white, nonhypercholesterolemic, and normotensive. Baseline measures of glucose, alkaline phosphatase, and liver function tests were all within normal limits.

Difference in cardiovascular disease risk factors by treatment diet are shown in Table 3. Significantly lower levels of serum cholesterol were observed in both soy diets compared with the carbohydrate comparison diet (6% decline). Similarly, LDL cholesterol was significantly reduced in the soy diets (7.5% lower). No significant differences were noted in HDL cholesterol. Compared with the carbohydrate diet, triglyceride levels were 28 mg/dL lower in the once-daily soy diet and 15 mg/dL lower in the twice-daily soy diet but these differences did not reach statistical significance. Figure 1 compares the lipid and lipoprotein levels among the treatment diets and illustrates the beneficial effect of the soy protein supplement on lipids and lipoproteins. No significant differences were observed in systolic blood pressure among the diets, whereas diastolic blood pressure levels were significantly lower in the twice-daily soy diet compared with the carbohydrate supplement diet (4.9 mm Hg lower), and no significant decline was noted with the once-daily soy diet. No significant differences were observed in weight change among the diets.

TABLE 2. *Baseline description of participants in the soy supplement crossover trial*

Variable ($n = 51$)	Mean	Standard deviation
Age (yrs)	51.0	4.8
Cholesterol (mg/dL)	208.0	40.5
LDL cholesterol (mg/dL)	126.9	38.5
HDL cholesterol (mg/dL)	54.9	15.6
Triglycerides (mg/dL)	131.1	67.1
Systolic blood pressure (mm Hg)	132.0	19.6
Diastolic blood pressure (mm Hg)	82.2	11.4
Weight (lbs)	163.4	38.2
Height (inches)	65.7	4.5
Fasting glucose (mg/dL)	91.7	20.6
Alkaline phosphatase	92.2	25.6
Blood urea nitrogen	13.0	3.8

LDL, low density lipoprotein; HDL, high density lipoprotein.

TABLE 3. *Mean differences in CVD risk factors by treatment group*

Variable[a]	Comparison group (carbohydrate supplement)		Soy group (once daily)		Soy group (twice daily)	
	Mean	(Std Error)	Mean	(Std error)	Mean	(Std error)
Cholesterol (mg/dL)	208.1	(2.6)	198.6	(2.6)[b]	195.7	(2.7)[b]
LDL cholesterol (mg/dL)	125.4	(2.3)	119.4	(2.3)[c]	116.8	(2.4)[b]
HDL cholesterol (mg/dL)	53.3	(0.9)	52.2	(0.9)	52.2	(0.9)
Triglycerides (mg/dL)	156.3	(12.3)	128.5	(13.0)	140.9	(12.7)
Systolic blood pressure (mm Hg)	126.6	(1.7)	124.2	(1.6)	125.3	(1.6)
Diastolic blood pressure (mm Hg)	78.6	(1.4)	76.3	(1.4)	73.7	(1.4)[b]
Weight (lbs)	164.2	(0.6)	164.6	(0.6)	164.8	(0.6)

CVD, cardiovascular disease; LDL, low density lipoprotein; HDL, high density lipoprotein; Std, standard.
[a]Adjusted for crossover trial treatment order.
[b]Significantly different from comparison group: $p < 0.01$.
[c]Significantly different from comparison group: $p < 0.05$.

Although the follow-up was for a relatively short period, we were interested to see if differences could be observed in quality of life and in menopausal symptoms (Table 4). An improvement in symptoms attributable to lack of estrogen was observed in both soy diet groups, and this difference reached statistical significance in the twice-daily soy diet compared with the carbohydrate diet. No significant differences were observed in the number of hot flushes or night sweats per week; however, hot flush severity was significantly lower in the split-dose soy diet. Night sweat severity improved slightly, although these differences did not reach statistical significance. In addition, a nonsignificant difference suggesting a beneficial effect of soy consumption on the general health score was observed. Of interest, we found that no differences in the presence of gastrointestinal symptoms were reported among the three diets. While the percent of expected packets consumed was highest in the carbohydrate diet, no significant differences were observed.

Differences in clinical chemistry laboratory variables by treatment group are shown in Table 5. Statistically higher levels of blood urea nitrogen were noted in both soy consumption diets, attributable to the increased protein intake in these groups. A significant decrease in alkaline phosphatase levels was present in both soy diets compared with the carbohydrate comparison group. No differences were observed in other liver function tests, serum protein, or fasting glucose levels.

DISCUSSION

These data provide evidence of a beneficial effect of soy protein supplementation on chronic disease risk factors in women consuming a typical Western diet. Soy protein supplementation was quite palatable and extremely well tolerated in these women.

Significant and substantial effects on lipids and lipoproteins were observed. Total plasma cholesterol levels were reduced by 6%, and LDL cholesterol levels were reduced by 7%. No significant effects were observed for triglycerides or HDL cholesterol. In contrast to estrogen replacement therapy,[20] soy protein does not increase triglyceride levels and may actually reduce levels, whereas traditional HRT has been shown to raise HDL cholesterol. These data confirm the beneficial effects of soy intake on lipids and lipoproteins noted by other investigators and summarized in a recent meta-analysis.[11–13] In fact, this study extends findings of previous investigations by documenting a significant effect on lipids/lipoproteins in nonhypercholesterolemic

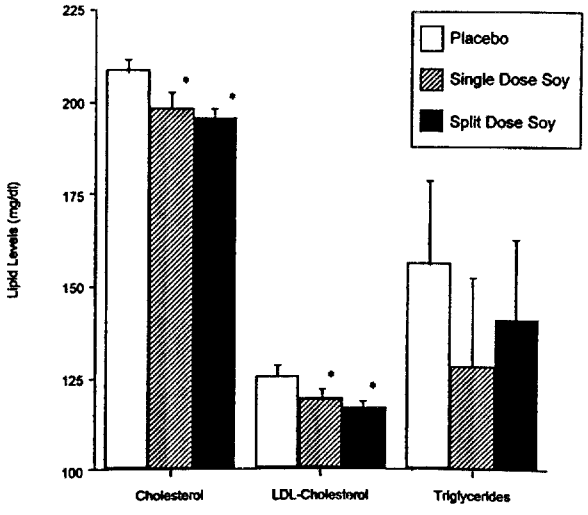

FIG. 1. Differences in lipids and lipoproteins between treatment groups adjusted.

TABLE 4. *Mean differences in adherence and reported symptoms by treatment group*

Variable[a]	Comparison group (carbohydrate supplement)		Soy group (once daily)		Soy group (twice daily)	
	Mean	(Std error)	Mean	(Std error)	Mean	(Std error)
Estrogenic symptom score	46.1	(0.7)	47.5	(0.7)	48.5	(0.7)[b]
Hot flashes (number/week)	21.3	(3.0)	23.1	(4.9)	22.3	(2.7)
Hot flash severity score	11.0	(0.4)	11.2	(0.7)	8.6	(0.4)[c]
Night sweats (number/week)	7.5	(0.4)	7.0	(0.6)	7.8	(0.3)
Night sweat severity score	6.2	(0.4)	5.9	(0.6)	5.5	(0.3)
General health score	67.9	(1.6)	72.2	(1.5)	71.7	(1.6)
Sleep disturbance score	35.0	(1.0)	34.6	(1.0)	36.9	(1.0)
GI symptom score	27.5	(0.6)	28.0	(0.5)	27.0	(0.6)
Percent of packets consumed (%)	96.9		94.3		93.4	

[a]Adjusted for crossover trial treatment order.
[b]Significantly different from comparison group: $p < 0.05$.
[c]Significantly different from comparison group: $p < 0.001$.

TABLE 5. *Mean differences in laboratory variables by treatment group*

Variable[a]	Comparison group (carbohydrate supplement)		Soy group (once daily)		Soy group (twice daily)	
	Mean	(Std error)	Mean	(Std error)	Mean	(Std error)
Fasting glucose (mg/dL)	91.8	(1.4)	92.6	(1.4)	92.3	(1.5)
Alkaline phosphatase	91.9	(1.3)	88.2	(1.3)[b]	88.3	(1.4)[b]
Blood urea nitrogen	12.4	(0.5)	13.7	(0.5)	13.9	(0.5)[b]
SGOT	25.8	(1.0)	24.6	(1.0)	24	(1.0)
LDH	176.1	(3.1)	174.7	(3.1)	174.3	(3.3)
Serum protein	7.1	(1.6)	7.6	(1.6)	10.3	(1.6)

SGOT, serum glutamic oxaloacetic transaminase; LDH, low density lipoprotein.
[a]Adjusted for crossover trial treatment order.
[b]Significantly different from comparison group: $p < 0.05$.

participants. Studies in animal models have shown similar effects on lipids and lipoproteins and have also shown a reduction in atherosclerosis.[21–23]

Of interest, based on mortality data from the lipid research clinics study, a 6% decrease in total cholesterol levels would be expected to reduce coronary heart disease risk by approximately 12%,[24] suggesting a substantial impact on coronary heart disease primary prevention from dietary soy supplementation.

Previous studies have suggested the potential for reduction of vasomotor symptoms with soy supplementation in peri- and postmenopausal women. A similar improvement in symptoms was observed in a study of menopausal women who received either wheat flour or soy flour.[14] A recent study observed a significant decline in the number of vasomotor symptoms in women consuming a higher dose of soy isoflavones.[15] In this study, we observed a significant improvement in the severity of menopausal symptoms and a significant improvement in hypoestrogenic related symptoms. While other studies have shown effects on the frequency of vasomotor symptoms, no changes were observed in this study. However, given the relatively short duration of these interventions (6 weeks) and the potential contamination inherent in a crossover trial, expecting larger effects on menopausal symptoms may be unrealistic. The phytoestrogens (genistein and diadzein) found in soy protein bind to estrogen receptors and have approximately 1000 to 10,000 × less estrogenic activity for the α receptors and approximately 3 × less estrogenic activity for the β receptor when compared with estradiol.[25] A definitive answer to the question of the efficacy of soy protein supplementation to cause substantial reductions of menopausal symptoms may be obtained only through longer term, randomized, double-blind clinical trials.[26]

Other effects of soy protein supplementation were observed in this trial. A beneficial effect on blood pressure levels was observed. Specifically, systolic blood pressure was 1.3 and 2.4 mm Hg lower with the soy protein diets compared with the carbohydrate comparison diet, whereas a 2.3 to 4.9 mm Hg decline in diastolic

blood pressure levels was observed. The effect on blood pressure levels was unexpected and requires confirmation in future studies. The increase in blood urea nitrogen was attributable to the increased protein intake and hence would not be expected to have any detrimental impact on participants with normal renal function. Lower levels of alkaline phosphatase were observed in the soy treatment groups. This may be a reflection of a beneficial effect of soy on this marker of bone metabolism. However, this result needs to be interpreted with caution because some differences in calcium and vitamin D levels were present between the carbohydrate and soy dietary supplements.

One of the unique aspects of this study was the ability to standardize the amount of phytoestrogens in the soy protein. Studies by our group in nonhuman primate models suggest that phytoestrogens are the components that mediate much of the beneficial chronic disease effects of soy.[23] The beneficial effects on plasma lipids and lipoproteins and blood pressure in this short study may indeed be related to the fact that significant levels of these compounds were available in the supplement. However, although this particular soy supplement was efficacious, it only contains approximately 50% of the average daily intake of phytoestrogens in the Japanese diet.[27,28] The fact that these changes were observed in women consuming a supplement in addition to their normal western diet has public health significance, because making similar dietary modifications to lower chronic disease risk requires only modest lifestyle changes.

The phytoestrogen concentration used in this trial is identical to the concentration normally found in soy protein. Based on our experience in this trial, consumption of this amount of soy protein had no short-term adverse consequences on any of the measured variables. Additionally, there is no a priori reason to anticipate any adverse outcomes, given that this food item is so commonly consumed around the world.

There were a number of limitations of this study. The relatively short duration (6-week intervention periods) of this study precluded assessment of effects that would be expected to occur over a longer time period. The relatively weak estrogenic potency of these compounds will require a longer study to fully address their effectiveness in relieving menopausal symptoms. In addition, this study could not address the hypothesized anticarcinogenic effect of soy supplementation. Specifically, other effects of these compounds such as tyrosine kinase inhibition have been postulated to cause lower rates of reproductive and other cancers in Asia and the Pacific rim compared with Western countries.[3,29,30] The design of the study has some inherent limitations. Because this study was a crossover clinical trial, it is possible that some contamination or carryover could have occurred between interventions and modified the outcome in the subsequent intervention. The carbohydrate placebo group differed from the soy groups for calcium (lower), sodium (lower), potassium (lower), and folate (lower) although these differences would not be expected to mediate the observed differences in outcomes between the groups. Despite the fact that this study had a larger sample size than previous investigations, it is possible we may have not observed some true associations because of lack of statistical power.

Although similar effects were noted in the single-dose and the split-dose soy supplementation diets, the effect on vasomotor symptoms was greater on the split-dose diet. This may suggest that having more consistent circulating levels of phytoestrogens may be more efficacious than a higher single dose. In addition, it is possible that higher doses of phytoestrogens than were used in this study may exert an even greater effect on these symptoms. To adequately address these important questions will require larger sample sizes, longer follow-up times, or a higher intake of phytoestrogens.

CONCLUSIONS

These data, along with previous reports, confirm the beneficial effects of soy protein supplementation on chronic disease risk in Western populations. Current dietary recommendations suggest replacing animal products with vegetable products to improve health.[31] Incorporation of soy into this dietary strategy will have beneficial effects on cardiovascular disease risk factors. Dietary soy supplementation may have the additional effect of attenuating menopausal symptoms. Thoughtful consideration of the addition of soy-based foods to a Western diet seems reasonable based on these and other data.

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